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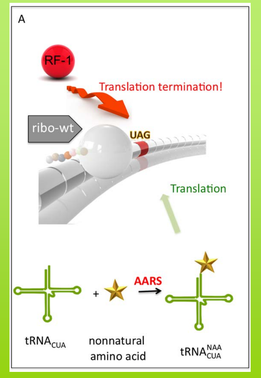

On the efficiency of an enzyme that can incorporate non-natural amino acids into proteins

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Introduction

Expanding the genetic code of an organism opens new avenues to modulate protein function. Interestingly, all known organisms use the same 20 common amino acids to make all the proteins on the planet- even though, genetically, we could sustain many more. Here, we altered an enzyme that naturally incorporated the common amino acid leucine into proteins. The alterations, or mutations, caused the enzyme to prefer a non-natural amino acid over its normal leucine partner. There have been more than 100 non-natural amino acids that have been successfully incorporated into proteins using this method, but the non-natural amino acid tested in this experiment is the only one that contains a metal. The focus of this project is to make the enzyme and test just how efficiently the enzyme incorporates the non-natural amino acid.

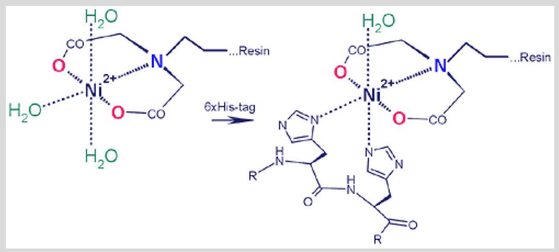

Figure 1. Leucyl aaRS binding to the 6xHis tag.

Methods and Results

The first goal was to obtain and purify the enzyme Leucyl amino acyl tRNA synthase (aaRS) found in E. coli. It was purified by using affinity chromatography. The desired enzyme binds to the 6xHis tag. Low concentrations of imidazole were run through to elute unwanted proteins and then a high concentration of imidazole is run through to collect the desired enzyme.

Methods and Results (cont.)

The purity of the leucyl aaRS enzyme collected was analyzed by using SDS-PAGE gel electrophoresis.

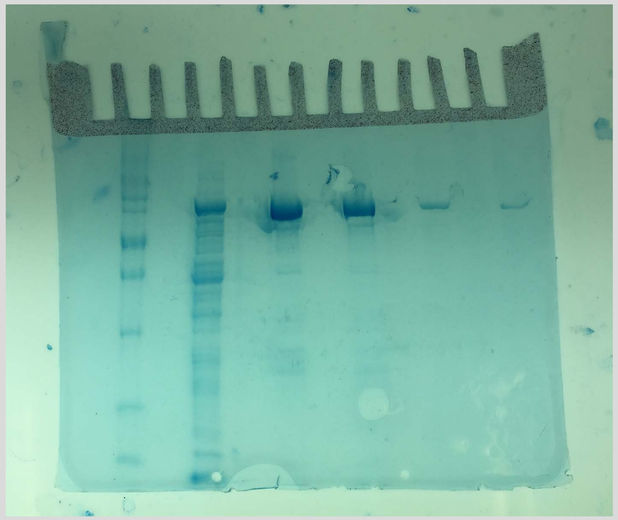

Figure 2. Lane 1 Molecular weight ladder; Lane 2 flow through; Lanes 3-6 fractions following Hexahistidine affinity purification.

The second goal was to determine how efficiently the purified enzyme reacts with the previously made non-natural amino acid ferrocenyl cysteine. The assay needed to do so must be validated so a different natural enzyme with known activity is used to prove the assay. The enzyme used is Tyr RS found in M. Jannaschii.

Summary

The overall goal of this research is to see how effectively the non-natural amino acid ferrocenyl cystein reacts with the enzyme leucyl amino acyl tRNA synthase. We want the enzyme to react efficiently with this non-natural amino acid even in the presence of its natural competitor leucine. By proving this interaction effective, we can insert this non-natural amino acid into proteins in order to alter their function. The ability to alter a protein's function can provide advances in both the medical and biochemical fields.

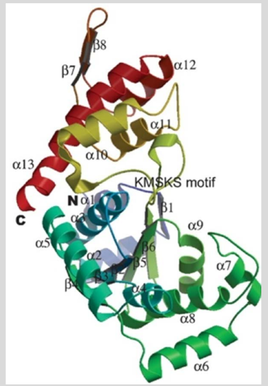

Figure 3. Tyr RS enzyme found in M. Jannaschii

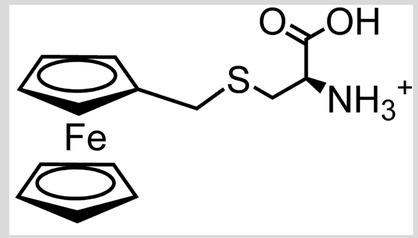

Figure 4. Structure of the non-natural amino acid ferrocenyl cysteine

Acknowledgements

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References

1. Wang et al. (2002) Science p. 498.
2. Tippmann and Schultz (2007) Tetrahedron p. 6182.
3. Tang and Tirrell (2002) Attenuation p. 10635